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Up and away: ontogenic transference as a pathway for aerial dispersal of microplastics

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Abstract

Microplastics (MPs) are ubiquitous pollutants found in marine, freshwater and terrestrial ecosystems. With so many MPs in aquatic systems it is inevitable that they will be ingested by aquatic organisms, and be transferred up through the food chain. However, to date, no study has considered whether MPs can be transmitted by means of ontogenic transference i.e. between life stages that utilise different habitats. Here, we determine whether fluorescent polystyrene beads could transfer between *Culex* mosquito life stages and, particularly, could move into the flying adult stage. We show for the first time that MPs can be transferred ontogenically from a feeding (larva) into a non-feeding (pupa) life stage and subsequently into the adult terrestrial life stage. However, transference is dependent on particle size, with smaller 2μm MPs transferring readily into pupae and adult stages, whilst 15μm MPs transferred at a significantly reduced rate. Microplastics appear to accumulate in the Malpighian tubule renal excretion system. The transfer of MPs to the adults represents a potential aerial pathway to contamination of new environments. Thus, any organism that feeds on terrestrial life phases of freshwater insects could be impacted by MPs found in aquatic ecosystems.

Keywords

Food chain: ontology; life stage; Malpighian tubules, microplastics; *Culex pipiens*

Introduction

Microplastics (MPs) are ubiquitous pollutants found in marine, freshwater and terrestrial ecosystems [1–3]. There is little doubt that plastic and MP pollution is a major environmental concern globally. Despite this, there is relatively little research into the impact of MPs on freshwater ecosystems, with most research concentrating on marine systems and organisms [2]. MPs have been defined as plastic particles smaller than 5mm in size [4,5]. However, this simple description covers a wide range of types, with polypropylene, polyethylene and polystyrene MPs entering the environment in different shapes and sizes, including fibres, pellets and cosmetic beads [6,7]. MPs are categorised based on their origin as primary or secondary types, depending on whether they were released into the environment as MPs (primary) or have degraded to that size in the environment (secondary) [8,9]. Microplastics pass through terrestrial environments in wastewater [2,10]. Rivers can subsequently deliver MPs into lakes, where they can be found in high concentrations [11–13].

Microplastics are ingested by aquatic organisms, and can be transferred through the food chain in both freshwater and marine environments [14–18]. However, to date no study has considered whether MPs can be transmitted by means of ontogenic transference i.e. between life stages that utilise different habitats. Freshwater environments are inhabited by insects that spend their juvenile stages in water but their adult stages in the terrestrial environment. Such insects include mayflies, dragonflies, midges and mosquitoes, most of which are eaten by terrestrial vertebrates. This raises the potential for MPs to enter terrestrial ecosystems from freshwater habitats aerially *via* transference to adult invertebrate life stages. Here, we thus determine whether 2 and 15μm fluorescent polystyrene beads could transfer between insect life stages and, particularly, could move into the flying adult stage. Fluorescent beads were selected to enable MPs to be easily detected in the non-feeding stages and also to allow an investigation of location within the body during metamorphosis. The *Culex pipiens* mosquito complex was selected as a model for this study given their worldwide distribution and broad habitat preference [19]. Mosquitoes develop through four feeding larval instars and a non-feeding pupal stage, and finally emerge into a flying adult.

Materials and methods

For additional details of all methods and analyses, see the electronic supplementary material.

Two types of MPs were used: a 2µm fluorescent yellow-green carboxylate-modified polystyrene (density 1.050g/cm^{3,} excitation 470nm; emission 505nm, Sigma-Aldrich, UK) and a 15.45$\pm$1.1µm fluorescent dragon green polystyrene (density 1.06 g/cm^3 (5x10^6 particles/ml, excitation 480nm; emission 520nm, Bangs Laboratories Inc., USA). Four treatments were used; a control with no microplastics, a treatment of 8x10^5 2µm particles/ml, a treatment of 8x10^2 15µm particles/ml, and a 1:1 mixture of both treatments. Each replicate (five per treatment) contained ten 3rd instar *C. pipiens* larvae in a 50ml glass beaker filled with 50ml of tap water. The control and all treatments contained 100mg of pelleted guinea pig food. Treatments were assigned randomly to a position on the laboratory bench to reduce experimental error.

One random individual was removed from each beaker when every mosquito had moulted into the 4th instar, and again when they pupated or emerged as adults. All samples were then placed in separate 1.5ml Eppendorf tubes and stored at -20 ºC prior to examination. Microplastics were extracted from mosquitoes by homogenization and filtration. The filter membrane was examined using an epi-fluorescent microscope (Zeiss Axioskop) under a 20x lens to count the number of fluorescent MPs. Adults were further dissected under a binocular stereo microscope (0.7X-4.5X) to extract the gut and quantify the numbers of MPs under the epi-fluorescent microscope [20].

All data were analyzed using the statistical software R v3.4.2 [21]. Microplastic counts were analysed using generalized linear models (GLMs) assuming a quasi-Poisson distribution. Uptake of microplastics was examined with respect to ‘particle size’, ‘treatment’ and ‘life stage’. We performed model simplification via stepwise removal of non-significant effects. Tukey tests were used post hoc for multiple comparisons.

Results

No MPs were found in control groups of any mosquito life stage. However, when exposed to MPs, particles were transferred from larvae to pupae, and subsequently to adults (Table S1). Significantly more 2µm particles were found in mosquito life stages than 15µm particles overall ($F_{1, 58}$=303.98, P<0.001). However, densities of MPs were significantly different between life stages ($F_{2, 56}$=160.42, P<0.001), with MP numbers significantly falling as mosquitoes moved between successive ontogenic levels (all P< 0.001). Microplastics uptake was also significantly greater overall in mixed exposure treatments ($F_{1, 55}$=6.00, P=0.02). Although 2µm particles were transferred to adults in all instances, we found no transference of 15µm particles following single treatment exposures. However, in the mixed MPs treatment, transference to adults of both 2µm and 15µm particles was evidenced (Figure 1). Microplastic ingestion was confirmed by fluorescent microscopy where the beads were detected in the adult abdomen, specifically inside the Malpighian tubules (Figure 2).

Discussion

Here, we show for the first time that MPs can be transferred ontogenically from a feeding (larval) into a non-feeding (pupal) life stage and subsequently into the flying (adult) life stage. Transference through to adults was found in both MP sizes, although the larger 15µm MPs were not ingested as readily as the 2µm MPs. Dissection of mosquito adults showed that 2µm

MPs accumulated in the renal excretion system of Malpighian tubules which, unlike the gut, pass from larvae to adult stages without visible reorganization [22]. This has been demonstrated previously to provide a physical transport system between stages during metamorphosis for *Pseudomonas* bacteria and seems to be important for ontogenic transmission from larvae to adults [23].

Few 15μm MPs were transferred into adults suggesting that MP size is an important factor in ontogenic transfer which could be related to the transfer and accumulation of MPs in the Malpighian tubes. Although the translocation mechanism of MPs to the Malpighian tubules is unclear in mosquitoes, analysis of fish, fiddler crab and marine mussels has demonstrated that MPs can be translocated from gastrointestinal tracts into other tissues in a wide range of phyla [24, 25,26]. Malpighian tubules have an entry point to the gut between the mid- and hindgut of mosquitoes, but the flow of fluid is from the Malpighian tubules to the hindgut [27]. Diptera are known to produce structures called concretions in the Malpighian tubules which have been shown to sequester heavy metals [28]. However, it is unlikely that this pathway would operate with a solid MP.

Our results have important implications since any aquatic life stage that is able to consume MPs and transfer them to their terrestrial life stage is a potential vector of MPs onto novel aerial and terrestrial habitats. Ingestion of MP-contaminated organisms by terrestrial organisms is not new [29]. Indeed, the widespread distribution of MPs in marine environments has meant that animals such as fish and shellfish sold for human consumption are contaminated with a range of plastics with a consequent transference of MPs between trophic levels [24]. Unlike MP fibres, which are common in the air and atmosphere, there has been no evidence for MPs being transported into the air [24]. We have demonstrated that species with both aquatic and terrestrial stages can harbour MPs through their life history, with aerial and terrestrial animals accordingly open to MP exposure.

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Figure legends

Figure 1: Mean (log count) uptake of microplastics following single 2µm/15µm and mixed 2µm/15µm exposures. Means are ±SE (n=5 per experimental group).

Figure 2. Epi-fluorescent microscope images showing fluorescent microplastic particles within (A) the abdomen of an adult mosquito before dissection, and (B) the abdominal Malpighian tubules following dissection.

Ethics

Ethics committee approval was not required.

Data accessibility

Data files are available in online supplementary material.

Author contribution

A.C. and R.A. designed the study, R.A. collected data and R.A. and R.N.C. analysed data and drafted the manuscript. AC revised and approved the manuscript.

Competing interests

We declare we have no competing interests.

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